



Our Docket No. 20040351.DIB

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES**

Re App : Carol W. Readhead et al. : July 26, 2006
S.N. : 10/008,385 : Art Unit 1632
Filed : November 12, 2001 : Examiner Joanne Hama
For : TRANSFECTION, STORAGE AND TRANSFER
OF MALE GERM CELLS FOR GENERATION
OF TRANSGENIC SPECIES & GENETIC THERAPIES

APPEAL BRIEF FOR THE APPELLANTS

Mail Stop APPEAL BRIEF - PATENTS
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

I. REAL PARTY IN INTEREST

The real parties in interest are Imperial College
Innovations Ltd., Exhibition Road, Sherfield Building, London,
United Kingdom SW7 2AZ, by virtue of mesne Assignments,
including Assignments from certain inventors and other earlier
assignees and Cedar-Sinai Medical Center, 8700 Beverly
Boulevard, Los Angeles, California 90048, by assignment from
inventor, Carol W. Readhead. The present application is a
division of Serial No. 09/191,920 filed November 13, 1998, now
Patent No. 6,316,692 which, in turn, claimed the benefit of
Provisional Application 60/065,825 filed November 14, 1997. By

virtue of being a division of what is now Patent No. 6,316,692, the Assignment of that application inures to the present application. That patent has some seventeen total assignments of record and a printout of the Patent Assignment Abstract of Title for that patent is attached hereto as Appendix A.

II. RELATED APPEALS AND INTERFERENCES

There are no other appeals or interferences known to the owner of the subject application, the owner's legal representatives, or the inventors which will directly affect or be directly affected by or should have a bearing on the Board of Patent Appeals and Interferences in the pending appeal to the present knowledge and belief of the undersigned.

III. STATUS OF THE CLAIMS

The present application was filed on November 12, 2001 as Serial No. 10/008,385 and has undergone several Office Actions and amendments. The original claims 1-134, originally filed in parent application 09/191,920, were canceled in a Preliminary Amendment in which claims 135-155 were added. Since then, claims 156-160 have been added and claims 136, 139 and 144 have been canceled. The Office Action from which this Appeal was taken was issued on July 26, 2005, an earlier Final Action dated April 11, 2005, having been vacated by the Examiner. An After-Final Amendment was submitted dated September 16, 2005 and an

Advisory Action was issued on October 13, 2005. The Advisory Action indicated that for purposes of appeal, the After-Final Amendment would be entered and contained a further explanation of how the newer amended claims would be rejected. On January 26, 2006, a Notice of Appeal was lodged for the present appeal in which claims 135, 137-138, 140-143 and 145-160 stand rejected and no claim has been allowed.

Thus, the present status of all the claims is as follows:

1-134. (Canceled)

135. (Rejected)

136. (Canceled)

137-138. (Rejected)

139. (Canceled)

140-143. (Rejected)

144. (Canceled)

145-160. (Rejected)

What Appellants believe to be a true copy of the claims presently under appeal appears in Appendix B attached to this Brief.

IV. STATUS OF AMENDMENTS

All amendments submitted in this application are believed to have been entered and are presently considered to be of record.

V. SUMMARY OF THE CLAIMED SUBJECT MATTER

The field of transgenics was initially developed to understand the action of a single gene in the context of the whole animal and phenomena of gene activities, expression and interaction. This technology has been used to produce models for various diseases in humans and other animals. Transgenic technology is amongst the most powerful tools available for the study of genetics and the understanding of genetic mechanisms and function. It is also being used to study the relationship between genes and diseases. Many diseases are caused by a single genetic defect or are the result of complex interaction genes and environmental agents. The understanding of such interactions is of prime importance for the development of therapies, such as gene therapy and drug therapies, and also treatments such as organ transplantation.

Transgenesis has played a part in improving breeds of non-human mammals such as livestock. Historically, transgenic animals have been produced almost exclusively by microinjection of the fertilized egg. The microinjected fertilized eggs are then transferred to the genital tract of a pseudopregnant female. The generation of transgenic animals by this technique is generally reproducible and, for this reason, little has been done to improve on it. That technique, however, requires large

numbers of fertilized eggs and so has proved to be quite inefficient in producing transgenic animals.

The present claimed invention relates to the field of transgenics and gene therapy and, more specifically, to an *in vitro* method for incorporating at least one polynucleotide including a desired trait into a male germ cell. By means of the claimed invention, male germ cells are transfected with at least one polynucleotide encoding a gene product in operable linkage with a promoter integrated in a viral vector which enables the polynucleotide to incorporate into the germ cell. Germ cells in which the polynucleotide is incorporated into the genome of the germ cell can then be isolated for further use such as being returned to the testes under suitable conditions where they will be spontaneously repopulated.

The application contains a lone independent claim, method claim 135, which enumerates four actions to a method for incorporating at least one polynucleotide encoding a desired trait into a male germ cell.

In part (a), a male germ cell selected from a group of non-human vertebrate species is obtained. Reference may be had to the specification, for example, at page 4, lines 6-9.

Thereafter, in the second action of the method (b), the selected male germ cell is transfected *in vitro* with at least

one polynucleotide encoding a gene product in operable linkage with a promoter which is included in a virus or virus-derived DNA. This is done in the presence of a gene delivery mixture including at least one transfecting agent and, optionally, a polynucleotide encoding a genetic selection marker. Reference is made to page 5, lines 5-15 and lines 22-25; also at page 10, lines 6; page 11, line 22; example 10, page 22, line 12; page 23, line 14.

This is followed by (c), allowing the polynucleotide encoding a gene product to be taken up by and released into the germ cell. This is described by material found throughout the specification, including page 5, lines 5-15.

Finally, in (d), the cells in which the polynucleotide has been successfully incorporated into the genome are selected for further use. Reference is made to page 4, lines 10-12; also in example 10 at page 23, lines 2-14, for example.

It is believed that the material in the dependent claims is also well-described throughout the specification, for example a listing of viruses can be found at page 10, lines 28-page 11, line 11 for claim 140. A listing of a group of non-human mammals which can be used as in claim 151 is found at page 12, lines 12-20. For claim 160, see, for example, page 14, line 21 and page 15, line 14.

It is believed that the claimed subject matter is adequately described for one to clearly interpret the claims and references any needed further descriptive material.

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

The statutory provision of 35 U.S.C. § 103(a) forms the sole legal basis for the rejection of claims 135, 137-138, 140-143 and 145-160. Two literature references are relied upon by the Examiner with respect to claims 135, 137-138, 140-143 and 145-159. They include:

Brinster and Zimmermann (PNAS, USA, 91:11298-11302, 1994)

Vogel and Sarver (Clinical Microbiology Reviews, 8:406-410, 1995)

With respect to claim 160, in addition to the above-cited references, the Examiner has added an additional reference in the form of a paper by Wivel and Walters (Science, 262:533-538, 1993).

In view of the foregoing, generally, the only issue to be decided on appeal is whether either of the combinations of references cited by the Examiner demonstrate that the subject matter of the present claims fails to meet the requirement for patentability imposed by 35 U.S.C. § 103.

In other words, should the rejection of claims 135, 137-138, 140-143 and 145-159 under 35 U.S.C. § 103(a) as being

unpatentable over Brinster and Zimmermann (1994) in view of Vogel and Sarver (1995); and the rejection of claim 160 under 35 U.S.C. § 103(a) as being unpatentable over Brinster and Zimmermann (1994) and Vogel and Sarver (1995) in view of Wivel and Walters (1993) stand?

VII. ARGUMENTS

A. Grouping of Claims

Appellants believe that each and every claim should stand or fall on its own merits and that the limitations of each should be considered separately. The rejections based on 35 U.S.C. § 103 have been applied to all of the claims. However, the dependent claims, when contrasted with independent claim 135, incorporate additional features which provide further support for their patentability. Accordingly, it is the belief of the Appellants that each and every claim should have the ability to stand or fall on its own merits and that the limitations of each should be considered separately. While the main thrust of the arguments will be directed to the independent claim at issue, the dependent claims add limitations and define combinations that should be considered on their own merits.

For example, many of the dependent claims require certain germ cells, viral vectors, retro viral vectors, transfecting agents, genetic selection markers, etc.

B. The Cited Art

(1) Brinster and Zimmermann (1994), (Brinster et al)

Brinster et al report that stem cells isolated from testis of donor male mice will repopulate sterile testis when injected into seminiferous tubules. They further merely suggest at page 11298, column 2:

"Because of the unique characteristics and potential of stem cell spermatogonia, the ability to recover these cells, manipulate them *in vitro*, and transfer them to another testis would provide a valuable technique to study the process of spermatogenesis. Furthermore, modifications of these cells prior to transfer could influence the development of eggs fertilized by spermatozoa arising from the altered stem cells. We describe here the requisite first step in this approach, a method to transplant testis cells from one male to another and we demonstrate that spermatogenesis occurs from donor cells in the recipient male." (emphasis added)

Another pertinent reference occurs at page 11301, second column, last paragraph:

"If spermatogonia can be cultured and manipulated -- e.g., via targeted homologous recombination of DNA

sequences -- and individual modified clones of cells can be selected in a manner similar to embryonic stem cells (18, 19), then these cells could be used to create mice with germline modifications." (emphases added).

The reference does not disclose or teach actual modification of the isolated stem cells and merely suggests the possible advantages of stem cell *in vitro* manipulation, as in the quote first above. With reference to the second quote, the suggestions seem somewhat speculative and there is clearly nothing contained in the reference that would suggest to one skilled in the art how this might be accomplished.

(2) Vogel and Sarver (1995), (Vogel et al)

The Vogel et al reference is directed to nucleic acid vaccines. The reference does teach that viral vectors, including retroviral vectors, provide an avenue for introducing foreign DNA into a cell using direct *in vivo* injection of nonreplicating retroviral particles and that "genes transferred via retroviral vectors are inserted into the host chromosome, thereby insuring the perpetuity of the genetic information in the target cells" (page 408, second column, first paragraph).

That reference, however, does not deal in any manner with *in vitro* manipulation of male germ cells. Furthermore, there is no teaching that there is a requirement for the nucleic acid to be in a viral vector or for it to integrate into the chromosome in order to produce an immune response.

(3) Wivel and Walters (1993), (Wivel et al)

This reference deals with gene modification and disease prevention and discusses medical and ethical perspectives. Wivel et al teach that there are some human genetic diseases which are candidates for genetic intervention in the manner of correcting or preventing genetic deficiencies. They describe human diseases that would be candidates for prevention by germ-line gene modification. These include Lesch-Nyhan syndrome, Tay-Sachs disease and Metachromatic Leukodystrophy.

C. Authorities and Arguments

The rejections under appeal here are all based on a plurality of references. In determining the propriety of a rejection under 35 U.S.C. § 103 based on a plurality of references, it is well settled that the obviousness of an invention cannot be established by combining the teachings of the several pieces prior art absent some teaching, suggestion or incentive in the art itself supporting the combination. See *In re Fine*, 837 F.2d 1071, 5 U.S.P.Q. 2d 1596 (Fed. Cir. 1988). A test for

obviousness is what the combined teachings of the references, taken as a whole, would have suggested to those having ordinary skill in the art. See *In re Kaslow*, 707 F.2d 1366, 217 U.S.P.Q. 1089 (Fed. Cir. 1983).

During the patent examination process, the U.S. Patent Office bears the initial burden of presenting a *prima facie* case of unpatentability. See *In re Oetiker*, 977 F.2d 1443, 24 U.S.P.Q. 2d 1443 (Fed. Cir. 1992). When the U.S. Patent Office fails to meet this burden, the appellant is entitled to the patent. However, when a *prima facie* case is made, the burden then shifts to the applicant to come forward with evidence and/or arguments supporting patentability to rebut the *prima facie* case. Patentability *vel non* is then determined on the entirety of the record, by a preponderance of the evidence and the weight of the argument. See *In re Paisecki*, 745 F.2d 1468, 223 U.S.P.Q. 785 (Fed. Cir. 1984).

The initial burden of establishing a *prima facie* case of obviousness thus rests upon the Examiner and that burden can only be satisfied by showing that objective teachings in the prior art or knowledge generally attributed to one of ordinary skill in the art would have led such an individual to combine the relevant teachings of the cited references. It is also well settled that it is error to reconstruct the appellants' claimed

invention from the prior art by using the appellants' claim as a "blueprint". When prior art references require selective combination to render a subsequent invention obvious, there must be some definitive reason for the combination to be made other than the hindsight obtained from the invention itself. See *Interconnect Planning Corp. v. Feil*, 774 F.2d 1132, 227 U.S.P.Q. 543 (Fed. Cir. 1985). "One cannot use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to depreciate the claimed invention." In re *Fine*, supra (Fed. Cir. 1988).

"To prevent the use of hindsight based on the invention to defeat patentability of the invention, this court requires the examiner to show a motivation to combine the references that create the case of obviousness". In re *Rouffet*, 149 F.3d 1350, 47 U.S.P.Q. 2d 1453 (Fed. Cir. 1998). "[T]he suggestion to combine requirement stands as a critical safeguard against hindsight analysis and rote application of the legal test for obviousness." In re *Rouffet*, supra.

In analyzing whether claimed subject is properly rejected under 35 U.S.C. § 103 based upon a combination of prior art references, two factors must be considered: (1) whether the prior art would have suggested to one of ordinary skill in the art that they should make the claimed composition or device, or

carry out the claimed process; and (2) whether the prior art would also have revealed that in so making or carrying out, those of ordinary skill would have a reasonable expectation of success. In re Vaeck 947 F.2d 488, 20 U.S.P.Q. 2d 1438 (Fed. Cir. 1991).

It has further long been well known and important to note that obviousness to try a particular approach or to undertake particular research does not make the results obvious. See, for example, in re Tomlinson et al 150 USPQ (CCPA 1966, 623).

For the reasons discussed and those given below, appellants will show that the prior art references cited by the Examiner do not suggest the invention as a whole claimed in the subject application.

Even assuming that the asserted combinations are proper, the claims of appellants' application define an invention that is believed to be neither taught nor suggested by the references relied upon by the Examiner. The inventors have probed the strengths and weaknesses of the prior art and discovered an improvement in versatility and simplicity that has escaped those who came before. This is indicative of unobviousness rather than obviousness. *Fromsen v. Anitec Printing Plates, Inc.*, 132 F.3d 1437, 45 U.S.P.Q. 2d. 1269 (Fed. Cir. 1997).

Brinster et al (1994) in view of Vogel et al (1995)

Based on the final rejection of July 26, 2005 and the Advisory Action dated October 13, 2005, it is the Examiner's position that the teaching of Brinster et al provides motivation for an artisan to use spermatogonia to introduce germ-line changes. Furthermore, the Examiner states at the bottom of page 3 of the Advisory Action that the fact that Brinster et al discussed the issue suggests reasonable expectation of success that one could arrive at the claimed invention. To this, the Examiner asserts that the motivation to the artisan to use retroviral vectors is provided by Vogel et al. Appellants believe this position cannot properly be sustained for reasons of record and for reasons presented here.

The disclosure of Brinster & Zimmermann provides no indication of how to introduce genetic material into male germ cells whereas, in contrast, the present claims are restricted to the use of viral vectors which allow the polynucleotide to incorporate into the germ cell. Brinster et al provide technical teaching only with respect to the transfer of stem cells isolated from testes of donor mice to repopulate sterile testes of recipient mice. There is no technical teaching of the transfection of the male germ cells, and certainly no suggestion that this be done using integrating viral vectors. In contrast,

to the extent that Brinster et al discloses anything of relevance, it suggests targeted homologous recombination of DNA sequences as evidenced in the second passage quoted above.

Further, as has been noted with respect to even that passage, the use of the words "if", "then", and "could" suggests mere speculation on the part of the authors as to future endeavors that could be tried and also indicate that they have no insight as to how this would be accomplished. At the time of the article, it was also clear that the culture of male germ cells was difficult and that it was difficult to retain the cells' integrity and male germ cell character in cultures.

Secondly, even if those skilled persons were to consider attempting to manipulate spermatogonia, they are specifically taught to do so "*via targeted homologous recombination of DNA sequences*". As is clear from Capecchi (1989, Trends in Genetics, 5:70-76; also cited by the Examiner), this is totally different from the use of integrating viruses (see Figure 5 of Capecchi on page 75 which highlights the differences in these approaches). In fact, the whole thrust of Capecchi is to allow gene targeting in embryonic stem (ES) cells which requires homologous recombination between DNA sequences residing in the chromosome and newly introduced DNA sequences (see Abstract on page 70 of Capecchi).

Thus, Brinster et al, to the extent that it may be relevant, suggests trying homologous recombination and not the use of viral vectors which integrate in the genome. Furthermore, Brinster et al discloses only *targeted homologous recombination*: integration of virus or virus-derived DNA is not targeted.

Thirdly, Brinster et al gives two specific references (References 18 and 19) for the genetic modification contemplated. Reference 18 is Capecchi (above) which is directed at gene targeting. Reference 19, Smithies (1993) Trends in Genetics 9, 112-116, (abstract cited and included as Appendix A attached) is a review of animal models of human genetic disease and, as can be seen from its abstract, relates to gene targeting in ES cells and not the use of viruses in male germ cells.

Additional evidence of record exists in other references previously cited by the Examiner and since withdrawn including Kim et al (Mol. Reprod. Dev., 1997, 46:515-526) and Bachiller et al (Mol. Reprod. Dev., 1991, 30:194-200).

The evidence of Kim et al is that using a standard transfection procedure, i.e., liposome/DNA complexes, spermatozoa cannot be transfected so that the DNA is

incorporated into their chromosome DNA (see page 519, column 1, lines 4-7).

The evidence of Bachiller et al is that when using a standard transfection procedure, i.e., liposome/DNA complexes, to transfect sperm, the sperm does not generate transgenic animals (see last sentence of the Abstract).

Thus, the evidence of record is that there is no reasonable expectation of success in doing what the Examiner alleges is an obvious thing to do.

The present claims, on the other hand, are restricted to the polynucleotide to be transfected into the male germ cells being comprised in a virus or virus-derived DNA and germ cells being selected in which the polynucleotide has incorporated into the genome.

This primary reference has been combined with Vogel et al which is said to teach retrovirus-mediated gene transfer and to teach using a retrovirus as a way of introducing a retroviral vector as a means of stably introducing a gene into the host chromosome. The Examiner further asserts that the integration using a retroviral vector insures perpetuity of the genetic information in the target cells.

In this regard, the appellants maintain their position that Vogel et al is in a technical field sufficiently different,

i.e., nucleic acid vaccines, from the technical field of Brinster et al and the present invention that the skilled artisan in the field of interest would not be led to consult it.

Even if the combination of Brinster et al and Vogel et al were proper, it does not add information that is more relevant to the patentability of the present claims. The Vogel et al reference is directed to nucleic acid vaccines which has nothing to do with making transgenic animals. The reference is believed to contain a possible inference to try an approach, but only in a different field.

Moreover, with respect to fundamental differences between what is taught or suggested by Vogel et al and any motivation to use viral DNA or viral derived DNA, it is noted at the outset that nucleic acid vaccines work by the nucleic acid being able to express a polypeptide which acts as an antigen in an immune response. There is no requirement for the nucleic acid to be in a viral vector or for it to integrate into the chromosome in order to do this. Thus, it is but one possible approach.

This is plain from the section entitled "Nucleic Acid Vaccine Development" on page 406 in which it is made clear that (1) DNA expression vectors in cationic lipid vesicles can be used or (2) naked plasmid DNA vectors. The work of Davis et al (Reference 5 on page 407, column 1) demonstrates that, at least

in some tissues (e.g. regenerating muscle), recombinant plasmid and adenoviral vectors (non-integrating) are superior to retroviral vectors (integrating).

Given the above, one can only conclude that there is nothing whatsoever in Vogel et al which gives any guidance as to which, if any, of these systems would be applicable to the genetic manipulation of male germ cells. This is not surprising since Vogel et al is in such an unrelated field.

Furthermore, it appears that the present rejections represent an analysis based on an impermissible hindsight reconstruction of the invention by hindsight. The Examiner has attempted to reconstruct the claimed invention not from a position of what the skilled person would do in modifying the teachings of Brinster et al (which, if anything, is to attempt to use homologous recombination on spermatogonial cells), but impermissibly using knowledge gleaned from the claimed invention and casting around to try to find a paper which "fills in the gap" of Brinster et al. Vogel et al cannot do this since, as discussed, it is in a different field (vaccines) and so would not be considered by the skilled person in any event. Furthermore, Vogel et al, in any event, discloses a range of ways in which one could potentially genetically manipulate cells without suggesting any particular one.

It should be remembered that the authors of Kim et al (which has been discussed previously), who presumably were aware of a desire that any genetic manipulation of the cells was carried to the next generation, used a liposome-mediated gene delivery approach. It is noteworthy that Kim et al was published in 1997, i.e., immediately before the claimed priority date and some 2-3 years later than Brinster et al, thus indicating again that the use of integrating viral vectors was not obvious to the skilled person.

Brinster et al (1994) and Vogel et al (1995) in view of Wivel et al (1993)

It is the Examiner's position that one skilled in the art at the time of filing the present application would have known that if germ-line modifications had applicability in humans, it would have had applicability in non-human animals because studies accomplished in humans are based on studies carried out in animals. The Appellants believe this rejection should not stand.

Wivel et al also relates to a different field making the combination of three pieces of art a combination from three different fields which clearly stretches the scope of what would reasonably have been consulted by the skilled artisan. Wivel et al relates to the introduction of foreign DNA into the

pronucleus of a zygote or into a four or eight-cell embryo or the use of embryonic stem cells. There is no reference at all to male germ cell modifications. In fact, on page 533, column 3, about half-way down, notes:

"It must be acknowledged, however, that at present most of the experimental work involves DNA transfer into one of the pronuclei of the zygote, the delivery of DNA into a four or eight-cell embryo by a vector, or the use of embryonic stem cells".

Given the above, the Appellants believe that the further combination of Wivel et al with Brinster et al and Vogel et al would not suggest the invention claimed in claim 160.

Appellants believe that the references have been compiled through hindsight and would not have been consulted in combination by anyone working in the art. In addition, at best, the references might suggest an approach to try and it appears that the Examiner has offered conclusions based on the references that the references themselves do not suggest in order to arrive at the present rejections.

It remains that the Appellants believe that the combination of references cited and the present rejections is not proper but, in any event, they believe their claims to represent a clear inventive step over that combination.

CONCLUSION

Appellants believe that the Examiner has not sustained the burden of establishing a *prima facie* case of obviousness, and, therefore, the rejections based on 35 U.S.C. § 103 should not stand.

Appellants are convinced that the present claims are patentable and it is respectfully requested that the final rejection of the Examiner be reversed and the claims be allowed.

Respectfully submitted,

NIKOLAI & MERSEREAU, P.A.

A handwritten signature in cursive script, appearing to read "C. G. Mersereau".

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CERTIFICATE OF MAILING

I hereby certify that the foregoing Appeal Brief for the Appellants in triplicate, Assignments on the Web (Appendix A) in triplicate, Claims (Appendix B) in triplicate, a Petition for a four-month extension of time, a check in the amount of \$1590.00 and a Transmittal Letter in application Serial No. 10/008,385, filed November 12, 2001, are being deposited with the U.S. Postal Service as First Class Mail in an envelope addressed to: Mail Stop APPEAL BRIEF - PATENTS, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, postage prepaid, on July 26, 2006.

A handwritten signature in cursive script, reading "Barbara L. Davis".

Barbara L. Davis
On Behalf of C. G. Mersereau

Date of Signature: July 26, 2006



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Patent Assignment Abstract of Title

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For pending or abandoned applications please consult USPTO staff.**

Total Assignments: 17

Patent #: 6316692 Issue Dt: 11/13/2001 Application #: 09191920 Filing Dt: 11/13/1998

Inventors: CAROL W. READHEAD, ROBERT WINSTON

Title: TRANSFECTION, STORAGE AND TRANSFER OF MALE GERM CELLS FOR GENERATION OF
TRANSGENIC SPECIES & GENETIC THERAPIES

Assignment: 1

Reel/Frame: 009599/0661

Recorded: 11/13/1998

Pages: 3

Conveyance: ASSIGNMENT OF ASSIGNORS INTEREST (SEE DOCUMENT FOR DETAILS).

Assignor: READHEAD, CAROL W.

Exec Dt: 01/08/1998

Assignee: CEDAR-SINAI MEDICAL CENTER8700 BEVERLY BOULEVARD
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Assignment: 2

Reel/Frame: 009599/0632

Recorded: 11/13/1998

Pages: 4

Conveyance: ASSIGNMENT OF ASSIGNORS INTEREST (SEE DOCUMENT FOR DETAILS).

Assignor: WINSTON, ROBERT

Exec Dt: 01/23/1998

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Assignment: 3

Reel/Frame: 015108/0726

Recorded: 03/22/2004

Pages: 6

Conveyance: ASSIGNMENT OF ASSIGNORS INTEREST (SEE DOCUMENT FOR DETAILS).

Assignor: HOVATTA, OUTI

Exec Dt: 03/03/2003

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Assignment: 4**Reel/Frame:** 015320/0238**Recorded:** 05/10/2004**Pages:** 8**Conveyance:** QUITCLAIM**Assignors:** WINSTON, PROFESSOR LORD ROBERT MAURICE
HAMMERSMITH HOSPITAL NHS TRUST**Exec Dt:** 03/31/2004**Exec Dt:** 03/31/2004**Assignee:** IMPERIAL COLLEGE INNOVATIONS LTD
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555 WEST FIFTH STREET, 50TH FLOOR
LOS ANGELES, CA 90013-1010**Assignment: 5****Reel/Frame:** 015320/0246**Recorded:** 05/17/2004**Pages:** 8**Conveyance:** ASSIGNMENT OF ASSIGNORS INTEREST (SEE DOCUMENT FOR DETAILS).**Assignor:** IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY AND **Exec Dt:** 03/31/2004
MEDICINE**Assignee:** IMPERIAL COLLEGE INNOVATIONS LTD
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555 WEST FIFTH STREET, 50TH FLOOR
LOS ANGELES, CALIFORNIA 90013-1010**Assignment: 6****Reel/Frame:** 015320/0267**Recorded:** 05/17/2004**Pages:** 5**Conveyance:** ASSIGNMENT OF ASSIGNORS INTEREST (SEE DOCUMENT FOR DETAILS).**Assignor:** IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY AND **Exec Dt:** 03/31/2004
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LOS ANGELES, CA 90013-1010**Assignment: 7****Reel/Frame:** 015361/0605**Recorded:** 05/24/2004**Pages:** 8**Conveyance:** ASSIGNMENT OF ASSIGNORS INTEREST (SEE DOCUMENT FOR DETAILS).

Assignor: IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY AND MEDICINE **Exec Dt:** 03/31/2004

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Assignment: 8

Reel/Frame: 015361/0613

Recorded: 05/17/2004

Pages: 8

Conveyance: QUITCLAIM

Assignors: WINSTON, LORD ROBERT MAURICE, PROFFESOR
HAMMERSMITH HOSPITAL NHS TRUST

Exec Dt: 03/31/2004

Exec Dt: 03/31/2004

Assignee: INPERIAL COLLEGE INNOVATIONS LTD
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Assignment: 9

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Recorded: 05/24/2004

Pages: 5

Conveyance: ASSIGNMENT OF ASSIGNORS INTEREST (SEE DOCUMENT FOR DETAILS).

Assignor: IMPERIAL COLLEGE OF SCIENCE TECHNOLOGY AND MEDICINE **Exec Dt:** 03/31/2004

Assignee: IMPERIAL COLLEGE INNOVATIONS LTD.
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Assignment: 10

Reel/Frame: 015361/0685

Recorded: 05/24/2004

Pages: 7

Conveyance: ASSIGNMENT OF ASSIGNORS INTEREST (SEE DOCUMENT FOR DETAILS).

Assignor: IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY AND MEDICINE **Exec Dt:** 03/31/2004

Assignee: IMPERIAL COLLEGE INNOVATIONS LTD.
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Assignment: 11**Reel/Frame:** 015361/0692**Recorded:** 05/24/2004**Pages:** 8**Conveyance:** QUITCLAIM**Assignors:** PROFESSOR LORD ROBERT MAURICE WINSTON
HAMMERSMITH HOSPITAL NHS TRUST**Exec Dt:** 03/31/2004**Exec Dt:** 03/31/2004**Assignee:** IMPERIAL COLLEGE INNOVATIONS, LTD.
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555 WEST FIFTH STREET, 50TH FLOOR
LOS ANGELES, CA 90013-1010**Assignment: 12****Reel/Frame:** 015361/0700**Recorded:** 05/24/2004**Pages:** 9**Conveyance:** QUITCLAIM**Assignors:** PROFESSOR LORD ROBERT MAURICE WINSTON
HAMMERSMITH HOSPITAL NHS TRUST**Exec Dt:** 03/31/2004**Exec Dt:** 03/31/2004**Assignee:** IMPERIAL COLLEGE INNOVATIONS, LTD.
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555 WEST FIFTH STREET, 50TH FLOOR
LOS ANGELES, CA 90013-1010**Assignment: 13****Reel/Frame:** 015361/0709**Recorded:** 05/24/2004**Pages:** 8**Conveyance:** ASSIGNMENT OF ASSIGNORS INTEREST (SEE DOCUMENT FOR DETAILS).**Assignor:** IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY AND **Exec Dt:** 03/31/2004
MEDICINE**Assignee:** IMPERIAL COLLEGE INNOVATIONS LTD
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555 WEST FIFTH STREET
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LOS ANGELES, CA 90013-1010**Assignment: 14****Reel/Frame:** 015361/0717**Recorded:** 05/24/2004**Pages:** 5**Conveyance:** ASSIGNMENT OF ASSIGNORS INTEREST (SEE DOCUMENT FOR DETAILS).**Assignor:** IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY AND **Exec Dt:** 03/31/2004
MEDICINE

Assignee: IMPERIAL COLLEGE INNOVATIONS LTD.
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Assignment: 15

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Pages: 5

Conveyance: ASSIGNMENT OF ASSIGNORS INTEREST (SEE DOCUMENT FOR DETAILS).

Assignor: IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY AND MEDICINE **Exec Dt:** 03/31/2004

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Assignment: 16

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Pages: 8

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Assignor: IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY AND MEDICINE **Exec Dt:** 03/31/2004

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Assignment: 17

Reel/Frame: 015361/0742

Recorded: 05/24/2004

Pages: 8

Conveyance: QUITCLAIM

Assignors: PROFESSOR LORD ROBERT MAURICE WINSTON
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Exec Dt: 03/31/2004

Exec Dt: 03/31/2004

Assignee: IMPERIAL COLLEGE INNOVATIONS LTD.
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Patent Assignment Abstract of Title

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Total Assignments: 17

Patent #: 6316692 Issue Dt: 11/13/2001 Application #: 09191920 Filing Dt: 11/13/1998

Inventors: CAROL W. READHEAD, ROBERT WINSTON

Title: TRANSFECTION, STORAGE AND TRANSFER OF MALE GERM CELLS FOR GENERATION OF TRANSGENIC SPECIES & GENETIC THERAPIES

Assignment: 1

Reel/Frame: 009599/0661

Recorded: 11/13/1998

Pages: 3

Conveyance: ASSIGNMENT OF ASSIGNORS INTEREST (SEE DOCUMENT FOR DETAILS).

Assignor: READHEAD, CAROL W.

Exec Dt: 01/08/1998

Assignee: CEDAR-SINAI MEDICAL CENTER

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Assignment: 2

Reel/Frame: 009599/0632

Recorded: 11/13/1998

Pages: 4

Conveyance: ASSIGNMENT OF ASSIGNORS INTEREST (SEE DOCUMENT FOR DETAILS).

Assignor: WINSTON, ROBERT

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Assignee: IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY & MEDICINE

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Assignment: 3

Reel/Frame: 015108/0726

Recorded: 03/22/2004

Pages: 6

Conveyance: ASSIGNMENT OF ASSIGNORS INTEREST (SEE DOCUMENT FOR DETAILS).

Assignor: HOVATTA, OUTI

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Assignee: IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY AND MEDICINE

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Assignment: 4**Reel/Frame:** 015320/0238**Recorded:** 05/10/2004**Pages:** 8**Conveyance:** QUITCLAIM**Assignors:** WINSTON, PROFESSOR LORD ROBERT MAURICE
HAMMERSMITH HOSPITAL NHS TRUST**Exec Dt:** 03/31/2004**Exec Dt:** 03/31/2004**Assignee:** IMPERIAL COLLEGE INNOVATIONS LTD
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555 WEST FIFTH STREET, 50TH FLOOR
LOS ANGELES, CA 90013-1010**Assignment: 5****Reel/Frame:** 015320/0246**Recorded:** 05/17/2004**Pages:** 8**Conveyance:** ASSIGNMENT OF ASSIGNORS INTEREST (SEE DOCUMENT FOR DETAILS).**Assignor:** IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY AND **Exec Dt:** 03/31/2004
MEDICINE**Assignee:** IMPERIAL COLLEGE INNOVATIONS LTD
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555 WEST FIFTH STREET, 50TH FLOOR
LOS ANGELES, CALIFORNIA 90013-1010**Assignment: 6****Reel/Frame:** 015320/0267**Recorded:** 05/17/2004**Pages:** 5**Conveyance:** ASSIGNMENT OF ASSIGNORS INTEREST (SEE DOCUMENT FOR DETAILS).**Assignor:** IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY AND **Exec Dt:** 03/31/2004
MEDICINE**Assignee:** IMPERIAL COLLEGE INNOVATIONS LTD.
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LOS ANGELES, CA 90013-1010**Assignment: 7****Reel/Frame:** 015361/0605**Recorded:** 05/24/2004**Pages:** 8**Conveyance:** ASSIGNMENT OF ASSIGNORS INTEREST (SEE DOCUMENT FOR DETAILS).

Assignor: IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY AND MEDICINE **Exec Dt:** 03/31/2004

Assignee: IMPERIAL COLLEGE INNOVATIONS LTD
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Assignment: 8

Reel/Frame: 015361/0613

Recorded: 05/17/2004

Pages: 8

Conveyance: QUITCLAIM

Assignors: WINSTON, LORD ROBERT MAURICE, PROFFESOR
HAMMERSMITH HOSPITAL NHS TRUST

Exec Dt: 03/31/2004

Exec Dt: 03/31/2004

Assignee: INPERIAL COLLEGE INNOVATIONS LTD
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Assignment: 9

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Recorded: 05/24/2004

Pages: 5

Conveyance: ASSIGNMENT OF ASSIGNORS INTEREST (SEE DOCUMENT FOR DETAILS).

Assignor: IMPERIAL COLLEGE OF SCIENCE TECHNOLOGY AND MEDICINE **Exec Dt:** 03/31/2004

Assignee: IMPERIAL COLLEGE INNOVATIONS LTD.
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Assignment: 10

Reel/Frame: 015361/0685

Recorded: 05/24/2004

Pages: 7

Conveyance: ASSIGNMENT OF ASSIGNORS INTEREST (SEE DOCUMENT FOR DETAILS).

Assignor: IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY AND MEDICINE **Exec Dt:** 03/31/2004

Assignee: IMPERIAL COLLEGE INNOVATIONS LTD.
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Assignment: 11**Reel/Frame:** 015361/0692**Recorded:** 05/24/2004**Pages:** 8**Conveyance:** QUITCLAIM**Assignors:** PROFESSOR LORD ROBERT MAURICE WINSTON
HAMMERSMITH HOSPITAL NHS TRUST**Exec Dt:** 03/31/2004**Exec Dt:** 03/31/2004**Assignee:** IMPERIAL COLLEGE INNOVATIONS, LTD.
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555 WEST FIFTH STREET, 50TH FLOOR
LOS ANGELES, CA 90013-1010**Assignment: 12****Reel/Frame:** 015361/0700**Recorded:** 05/24/2004**Pages:** 9**Conveyance:** QUITCLAIM**Assignors:** PROFESSOR LORD ROBERT MAURICE WINSTON
HAMMERSMITH HOSPITAL NHS TRUST**Exec Dt:** 03/31/2004**Exec Dt:** 03/31/2004**Assignee:** IMPERIAL COLLEGE INNOVATIONS, LTD.
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555 WEST FIFTH STREET, 50TH FLOOR
LOS ANGELES, CA 90013-1010**Assignment: 13****Reel/Frame:** 015361/0709**Recorded:** 05/24/2004**Pages:** 8**Conveyance:** ASSIGNMENT OF ASSIGNORS INTEREST (SEE DOCUMENT FOR DETAILS).**Assignor:** IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY AND **Exec Dt:** 03/31/2004
MEDICINE**Assignee:** IMPERIAL COLLEGE INNOVATIONS LTD
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555 WEST FIFTH STREET
50TH FLOOR
LOS ANGELES, CA 90013-1010**Assignment: 14****Reel/Frame:** 015361/0717**Recorded:** 05/24/2004**Pages:** 5**Conveyance:** ASSIGNMENT OF ASSIGNORS INTEREST (SEE DOCUMENT FOR DETAILS).**Assignor:** IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY AND **Exec Dt:** 03/31/2004
MEDICINE

Assignee: IMPERIAL COLLEGE INNOVATIONS LTD.

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Assignment: 15

Reel/Frame: 015361/0722

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Pages: 5

Conveyance: ASSIGNMENT OF ASSIGNORS INTEREST (SEE DOCUMENT FOR DETAILS).

Assignor: IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY AND MEDICINE **Exec Dt:** 03/31/2004

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Assignment: 16

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Recorded: 05/24/2004

Pages: 8

Conveyance: ASSIGNMENT OF ASSIGNORS INTEREST (SEE DOCUMENT FOR DETAILS).

Assignor: IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY AND MEDICINE **Exec Dt:** 03/31/2004

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Assignment: 17

Reel/Frame: 015361/0742

Recorded: 05/24/2004

Pages: 8

Conveyance: QUITCLAIM

Assignors: PROFESSOR LORD ROBERT MAURICE WINSTON

Exec Dt: 03/31/2004

HAMMERSMITH HOSPITAL NHS TRUST

Exec Dt: 03/31/2004

Assignee: IMPERIAL COLLEGE INNOVATIONS LTD.

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Total Assignments: 17

Patent #: [6316692](#) Issue Dt: 11/13/2001 Application #: 09191920 Filing Dt: 11/13/1998

Inventors: CAROL W. READHEAD, ROBERT WINSTON

Title: TRANSFECTION, STORAGE AND TRANSFER OF MALE GERM CELLS FOR GENERATION OF TRANSGENIC SPECIES & GENETIC THERAPIES

Assignment: 1

Reel/Frame: [009599/0661](#)

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Pages: 3

Conveyance: ASSIGNMENT OF ASSIGNORS INTEREST (SEE DOCUMENT FOR DETAILS).

Assignor: [READHEAD, CAROL W.](#)

Exec Dt: 01/08/1998

Assignee: [CEDAR-SINAI MEDICAL CENTER](#)

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Assignment: 2

Reel/Frame: [009599/0632](#)

Recorded: 11/13/1998

Pages: 4

Conveyance: ASSIGNMENT OF ASSIGNORS INTEREST (SEE DOCUMENT FOR DETAILS).

Assignor: [WINSTON, ROBERT](#)

Exec Dt: 01/23/1998

Assignee: [IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY & MEDICINE](#)

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Assignment: 3

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Pages: 6

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Assignor: [HOVATTA, OUTI](#)

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HAMMERSMITH HOSPITAL NHS TRUST**Exec Dt:** 03/31/2004**Exec Dt:** 03/31/2004**Assignee:** IMPERIAL COLLEGE INNOVATIONS LTD
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555 WEST FIFTH STREET, 50TH FLOOR
LOS ANGELES, CA 90013-1010**Assignment: 5****Reel/Frame:** 015320/0246**Recorded:** 05/17/2004**Pages:** 8**Conveyance:** ASSIGNMENT OF ASSIGNORS INTEREST (SEE DOCUMENT FOR DETAILS).**Assignor:** IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY AND **Exec Dt:** 03/31/2004
MEDICINE**Assignee:** IMPERIAL COLLEGE INNOVATIONS LTD
SHERFIELD BUILDING
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LONDON, UNITED KINGDOM SW7 2AZ**Correspondent:** SIDLEY AUSTIN BROWN & WOOD, LLP
EDWARD G. POPLAWSKI, ESQ.
555 WEST FIFTH STREET, 50TH FLOOR
LOS ANGELES, CALIFORNIA 90013-1010**Assignment: 6****Reel/Frame:** 015320/0267**Recorded:** 05/17/2004**Pages:** 5**Conveyance:** ASSIGNMENT OF ASSIGNORS INTEREST (SEE DOCUMENT FOR DETAILS).**Assignor:** IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY AND **Exec Dt:** 03/31/2004
MEDICINE**Assignee:** IMPERIAL COLLEGE INNOVATIONS LTD.
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555 WEST FIFTH STREET
50TH FLOOR
LOS ANGELES, CA 90013-1010**Assignment: 7****Reel/Frame:** 015361/0605**Recorded:** 05/24/2004**Pages:** 8**Conveyance:** ASSIGNMENT OF ASSIGNORS INTEREST (SEE DOCUMENT FOR DETAILS).

Assignor: IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY AND MEDICINE **Exec Dt:** 03/31/2004

Assignee: IMPERIAL COLLEGE INNOVATIONS LTD
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Assignment: 8

Reel/Frame: 015361/0613

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Conveyance: QUITCLAIM

Assignors: WINSTON, LORD ROBERT MAURICE, PROFFESOR
HAMMERSMITH HOSPITAL NHS TRUST

Exec Dt: 03/31/2004

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Assignee: INPERIAL COLLEGE INNOVATIONS LTD
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Assignment: 9

Reel/Frame: 015361/0680

Recorded: 05/24/2004

Pages: 5

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Assignor: IMPERIAL COLLEGE OF SCIENCE TECHNOLOGY AND MEDICINE **Exec Dt:** 03/31/2004

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Assignment: 10

Reel/Frame: 015361/0685

Recorded: 05/24/2004

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Assignment: 11**Reel/Frame:** 015361/0692**Recorded:** 05/24/2004**Pages:** 8**Conveyance:** QUITCLAIM**Assignors:** PROFESSOR LORD ROBERT MAURICE WINSTON**Exec Dt:** 03/31/2004HAMMERSMITH HOSPITAL NHS TRUST**Exec Dt:** 03/31/2004**Assignee:** IMPERIAL COLLEGE INNOVATIONS, LTD.

SHERFIELD BUILDING

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Assignment: 12**Reel/Frame:** 015361/0700**Recorded:** 05/24/2004**Pages:** 9**Conveyance:** QUITCLAIM**Assignors:** PROFESSOR LORD ROBERT MAURICE WINSTON**Exec Dt:** 03/31/2004HAMMERSMITH HOSPITAL NHS TRUST**Exec Dt:** 03/31/2004**Assignee:** IMPERIAL COLLEGE INNOVATIONS, LTD.

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Assignment: 13**Reel/Frame:** 015361/0709**Recorded:** 05/24/2004**Pages:** 8**Conveyance:** ASSIGNMENT OF ASSIGNORS INTEREST (SEE DOCUMENT FOR DETAILS).**Assignor:** IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY AND MEDICINE **Exec Dt:** 03/31/2004**Assignee:** IMPERIAL COLLEGE INNOVATIONS LTD

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Assignment: 14**Reel/Frame:** 015361/0717**Recorded:** 05/24/2004**Pages:** 5**Conveyance:** ASSIGNMENT OF ASSIGNORS INTEREST (SEE DOCUMENT FOR DETAILS).**Assignor:** IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY AND MEDICINE **Exec Dt:** 03/31/2004

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Assignment: 15

Reel/Frame: 015361/0722

Recorded: 05/24/2004

Pages: 5

Conveyance: ASSIGNMENT OF ASSIGNORS INTEREST (SEE DOCUMENT FOR DETAILS).

Assignor: IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY AND MEDICINE **Exec Dt:** 03/31/2004

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Assignment: 16

Reel/Frame: 015361/0734

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Conveyance: ASSIGNMENT OF ASSIGNORS INTEREST (SEE DOCUMENT FOR DETAILS).

Assignor: IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY AND MEDICINE **Exec Dt:** 03/31/2004

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Assignment: 17

Reel/Frame: 015361/0742

Recorded: 05/24/2004

Pages: 8

Conveyance: QUITCLAIM

Assignors: PROFESSOR LORD ROBERT MAURICE WINSTON
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APPENDIX B

CLAIMS

The following represents the current status of all the claims in the present application including changes made by this paper:

1-134 (canceled).

135 (previously presented). An in vitro method of incorporating at least one polynucleotide encoding a desired trait into a male germ cell, comprising:

- (a) obtaining a male germ cell from a non-human vertebrate species, said germ cell being selected from the group consisting of spermatogonial stem cells, type B spermatogonia, primary spermatocytes, preleptotene spermatocytes, leptotene spermatocytes, zygotene spermatocytes, pachytene spermatocytes, secondary spermatocytes, spermatids, and spermatozoa;
- (b) transfecting the germ cell in vitro with at least one polynucleotide encoding a gene product in operable linkage with a promoter comprised in a virus or virus-derived DNA, in the presence of a gene delivery mixture comprising at least one transfecting agent, and optionally a polynucleotide encoding a genetic selection

marker;

- (c) allowing the polynucleotide encoding a gene product to be taken up by, and released into the germ cell; and
- (d) selecting those cells in which the polynucleotide has incorporated into the genome of the germ cell.

136(canceled).

137(previously presented). The method of claim 135, wherein the male germ cell is selected from the group consisting of spermatogonial cells and other undifferentiated male germ cells.

138(previously presented). The method of claim 135, wherein the transfection is conducted under conditions of temperature of about 25°C to about 38°C.

139(canceled).

140(previously presented). The method of claim 135, wherein the viral vector is selected from the group consisting of retroviral vectors, adenoviral vectors, transferrin-polylysine enhanced adenoviral vectors, Moloney murine leukemia virus-derived vectors, mumps vectors, and virus-derived DNAs that enhance polynucleotide uptake by and release into the cytoplasm of germ cells or a mixture of any members of said group.

141(previously presented). The method of claim 140, wherein the retroviral vector is selected from the group consisting of lentiviral vectors.

142(previously presented). The method of claim 135, wherein the transfecting agent comprises an adenovirus vector having endosomal lytic activity, and the polynucleotide encoding a gene product is operatively linked to the vector.

143(previously presented). The method of claim 135, wherein the polynucleotide encoding a gene product is in the form of a complex with a viral vector.

144(canceled).

145(previously presented). The method of claim 135, wherein:

the transfecting agent further comprises an agent selected from the group consisting of a c-kit ligand and at least one genetic selection marker; and

the method further comprises isolating or selecting a male germ cell carrying at least one polynucleotide encoding a gene product at least one polynucleotide encoding a genetic selection marker, from a donor male vertebrate with the aid of the genetic selection marker.

146(previously presented). The method of claim 145, wherein the genetic selection marker comprises a gene

expressing a detectable product, driven by a promoter selected from the group consisting of c-kit promoters, b-Myb promoters, c-raf-1 promoters, ATM (ataxia-telangiectasia) promoters, RBM (ribosome binding motif) promoters, DAZ (deleted in azoospermia) promoters, XRCC-1 promoters, HSP 90 (heat shock gene) promoters, and FRMI (from fragile X site) promoters.

147(previously presented). The method of claim 135, wherein the non-human vertebrate is a mammal.

148(previously presented). The method of claim 147, wherein the mammal is selected from the group consisting of non-human primates and farm and marine mammals.

149(previously presented). The method of claim 135, wherein the polynucleotide encoding a gene product is derived from the same non-human vertebrate species as the germ cell.

150(previously presented). The method of claim 135, wherein the non-human vertebrate is selected from the group consisting of wild and domesticated vertebrates.

151(previously presented). The method of claim 135, wherein the polynucleotide encoding a gene product is derived from a non-human mammal selected from the group consisting of human and non-human primates, canines, felines, swines, farm mammals, pachyderms, marine mammals,

equines, murines, ovines and bovines, or from a bird selected from the group consisting of ducks, geese, turkeys and chickens.

152(previously presented). The method of claim 151, wherein the polynucleotide is derived from a human.

153(previously presented). The method of claim 135, wherein the promoter is a germ cell-specific promoter.

154(previously presented). The method of claim 135, wherein the polynucleotide encoding a genetic selection marker is operatively linked to a germ cell-specific promoter.

155(previously presented). The method of claim 145, wherein the polynucleotide encoding a genetic selection marker is operatively linked to a germ cell-specific promoter.

156(previously presented). The method of claim 135 including a further step of introducing transfected cells selected in step (d) into a testis of a male of a non-human vertebrate of the species from which said male germ cell was obtained, thereby producing injected males.

157(previously presented). The method of claim 156 wherein said transfected cells are injected into said testis via vasa efferentia.

158(previously presented). The method of claim 156

including a further step of breeding one or more of said injected males to one or more normal females to thereby produce transgenic non-human mammal progeny.

159(previously presented). A transgenic non-human mammal produced by the method of claim 158.

160(previously presented). The method of claim 135 wherein said at least one polynucleotide encoding a gene product in step (b) is one which is able to correct a gene disorder.

APPENDIX B

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The following represents the current status of all the claims in the present application including changes made by this paper:

1-134(canceled).

135(previously presented). An in vitro method of incorporating at least one polynucleotide encoding a desired trait into a male germ cell, comprising:

- (a) obtaining a male germ cell from a non-human vertebrate species, said germ cell being selected from the group consisting of spermatogonial stem cells, type B spermatogonia, primary spermatocytes, preleptotene spermatocytes, leptotene spermatocytes, zygotene spermatocytes, pachytene spermatocytes, secondary spermatocytes, spermatids, and spermatozoa;
- (b) transfecting the germ cell in vitro with at least one polynucleotide encoding a gene product in operable linkage with a promoter comprised in a virus or virus-derived DNA, in the presence of a gene delivery mixture comprising at least one transfecting agent, and optionally a polynucleotide encoding a genetic selection

marker;

- (c) allowing the polynucleotide encoding a gene product to be taken up by, and released into the germ cell; and
- (d) selecting those cells in which the polynucleotide has incorporated into the genome of the germ cell.

136(canceled).

137(previously presented). The method of claim 135, wherein the male germ cell is selected from the group consisting of spermatogonial cells and other undifferentiated male germ cells.

138(previously presented). The method of claim 135, wherein the transfection is conducted under conditions of temperature of about 25°C to about 38°C.

139(canceled).

140(previously presented). The method of claim 135, wherein the viral vector is selected from the group consisting of retroviral vectors, adenoviral vectors, transferrin-polylysine enhanced adenoviral vectors, Moloney murine leukemia virus-derived vectors, mumps vectors, and virus-derived DNAs that enhance polynucleotide uptake by and release into the cytoplasm of germ cells or a mixture of any members of said group.

141(previously presented). The method of claim 140, wherein the retroviral vector is selected from the group consisting of lentiviral vectors.

142(previously presented). The method of claim 135, wherein the transfecting agent comprises an adenovirus vector having endosomal lytic activity, and the polynucleotide encoding a gene product is operatively linked to the vector.

143(previously presented). The method of claim 135, wherein the polynucleotide encoding a gene product is in the form of a complex with a viral vector.

144(canceled).

145(previously presented). The method of claim 135, wherein:

the transfecting agent further comprises an agent selected from the group consisting of a c-kit ligand and at least one genetic selection marker; and

the method further comprises isolating or selecting a male germ cell carrying at least one polynucleotide encoding a gene product at least one polynucleotide encoding a genetic selection marker, from a donor male vertebrate with the aid of the genetic selection marker.

146(previously presented). The method of claim 145, wherein the genetic selection marker comprises a gene

expressing a detectable product, driven by a promoter selected from the group consisting of c-kit promoters, b-Myb promoters, c-raf-1 promoters, ATM (ataxia-telangiectasia) promoters, RBM (ribosome binding motif) promoters, DAZ (deleted in azoospermia) promoters, XRCC-1 promoters, HSP 90 (heat shock gene) promoters, and FRMI (from fragile X site) promoters.

147(previously presented). The method of claim 135, wherein the non-human vertebrate is a mammal.

148(previously presented). The method of claim 147, wherein the mammal is selected from the group consisting of non-human primates and farm and marine mammals.

149(previously presented). The method of claim 135, wherein the polynucleotide encoding a gene product is derived from the same non-human vertebrate species as the germ cell.

150(previously presented).. The method of claim 135, wherein the non-human vertebrate is selected from the group consisting of wild and domesticated vertebrates.

151(previously presented). The method of claim 135, wherein the polynucleotide encoding a gene product is derived from a non-human mammal selected from the group consisting of human and non-human primates, canines, felines, swines, farm mammals, pachyderms, marine mammals,

equines, murines, ovines and bovines, or from a bird selected from the group consisting of ducks, geese, turkeys and chickens.

152(previously presented). The method of claim 151, wherein the polynucleotide is derived from a human.

153(previously presented). The method of claim 135, wherein the promoter is a germ cell-specific promoter.

154(previously presented). The method of claim 135, wherein the polynucleotide encoding a genetic selection marker is operatively linked to a germ cell-specific promoter.

155(previously presented). The method of claim 145, wherein the polynucleotide encoding a genetic selection marker is operatively linked to a germ cell-specific promoter.

156(previously presented). The method of claim 135 including a further step of introducing transfected cells selected in step (d) into a testis of a male of a non-human vertebrate of the species from which said male germ cell was obtained, thereby producing injected males.

157(previously presented). The method of claim 156 wherein said transfected cells are injected into said testis via vasa efferentia.

158(previously presented). The method of claim 156

including a further step of breeding one or more of said injected males to one or more normal females to thereby produce transgenic non-human mammal progeny.

159(previously presented). A transgenic non-human mammal produced by the method of claim 158.

160(previously presented). The method of claim 135 wherein said at least one polynucleotide encoding a gene product in step (b) is one which is able to correct a gene disorder.

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- (b) transfecting the germ cell in vitro with at least one polynucleotide encoding a gene product in operable linkage with a promoter comprised in a virus or virus-derived DNA, in the presence of a gene delivery mixture comprising at least one transfecting agent, and optionally a polynucleotide encoding a genetic selection

marker;

- (c) allowing the polynucleotide encoding a gene product to be taken up by, and released into the germ cell; and
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the method further comprises isolating or selecting a male germ cell carrying at least one polynucleotide encoding a gene product at least one polynucleotide encoding a genetic selection marker, from a donor male vertebrate with the aid of the genetic selection marker.

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147(Previously presented). The method of claim 135, wherein the non-human vertebrate is a mammal.

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